Evaluation of *Dasypyrum villosum* Populations for Resistance to Cereal Eyespot and Stripe Rust Pathogens

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ABSTRACT

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Resistance to *Pseudocercosporella herpotrichoides* (cause of eyespot) and *Puccinia striiformis* (cause of stripe rust) was evaluated in a germ plasm collection of *Dasypyrum villosum* (syn. *Haynaldia villosa*) and a set of disomic addition lines, a substitution, and a translocation line of *D. villosum* chromosomes in a wheat background. Three races of *P. striiformis* and a β-glucuronidase-transformed strain of *Pseudocercosporella herpotrichoides* were used to inoculate plants and evaluate disease reactions. Of the 115 *D. villosum* accessions tested, 33 (28.6%) were resistant to one or more races of *Puccinia striiformis* and 8 accessions were resistant to all races. All 219 accessions of *D. villosum* tested were resistant to *Pseudocercosporella herpotrichoides* and 158 (72%) of the accessions had lower β-glucuronidase activity than the resistant wheat line VPM-1. Most of the accessions of *D. villosum* resistant to the stripe rust pathogen originated from Greece; however, there was no distinction among origins for resistance to the eyespot pathogen. Chromosome 4V was confirmed to carry the gene for resistance to *P. herpotrichoides*. At least one gene for resistance to *Puccinia striiformis* was located on the short arm of chromosome 6V of *D. villosum* in the 6VS/6AL-translocation line; this gene was named *Yr26*.

Dasypyrum villosum Candargy (syn. *Haynaldia villosa*) is a cross-pollinating, diploid ($2n = 2x = 14$) annual species that belongs to the tribe Triticeae (18). It is native to Southern Europe and West Asia, especially the Caucasuses (14), and grows under conditions unfavorable to most cultivated crops.

The genome of *D. villosum*, designated V by Sears (40), is considered an important donor of genes to wheat for improving powdery mildew resistance (6,13), take-all (39), eyespot (35,43), and plant and seed storage protein content (13,15,41). *D. villosum* has been hybridized with diploid, tetraploid, and hexaploid species of *Triticum* (3,6,15,19,21,38,40), and six of the seven *D. villosum* chromosomes have been added disomically to a hexaploid wheat background (40). Interspecific hybridization reveals very little homology between wheat and *D. villosum* and, until recently, it was believed that homologous gene transfer was not possible because V genome chromosomes do not pair well with wheat chromosomes (19,40). However, Chen et al. (6) have successfully recovered genotypes containing spontaneous translocations between wheat and *D. villosum* chromosome 6V with resistance to powdery mildew. Pollen fertility appears to be the main problem in crosses performed between *D. villosum* and wheat (4). F_1 plants are completely male sterile and partially female fertile (28). However, the extra effort required in gene transfer from *D. villosum* to wheat for eyespot resistance is warranted because of the small pool of resistance genes available to breeders (24).

Eyespot is one of the most economically important diseases of wheat in the U.S. Pacific Northwest and many other temperate wheat-growing areas in the world (23,44). The disease is caused by *Pseudocercosporella herpotrichoides* (teleomorph *Tapesia yallundae*) and affects the stem base of wheat and other grasses, causing weak stems, lodging at the seed filling stage, and production of grainless white heads (23). The disease has been controlled primarily with fungicides, but the widespread occurrence of fungicide resistance in the pathogen (35) has encouraged development of resistant cultivars. Breeders have successfully improved resistance in some commercial cultivars (1,2,16,25,32), but the continuing effort to improve resistance has been slow, because neither of the two known resistance genes offer complete resistance (24). One of the genes, *Pch1*, originated from the goat grass species *Aegilops ventricosa*, and is located on chromosome 7DL (17,20,29,42). The other gene, *Pch2*, comes from wheat cv. Cappelle Desprez and is located on chromosome 7AL (12,26). *Pch1* is the most widely utilized source of eyespot resistance in breeding programs, primarily because it is more effective than *Pch2*.

D. villosum is one of the wild wheats that was initially reported by Sprague (43) to be resistant to eyespot, but has been used little since that report. Recently, Murray et al. (35) located a new resistance gene for eyespot on chromosome 4V of *D. villosum*. Characterization and transfer of these new genes into hexaploid wheat may provide a more complete resistance to eyespot by pyramiding resistance genes into a single cultivar.

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is also an economically important disease of wheat in the Pacific Northwest and worldwide (27). Stripe rust is principally a destructive disease of wheat during the winter and early spring, causing up to 90% yield loss when the environment is favorable (37). On average, stripe rust epidemics occur three out of four years in the Pacific Northwest (10). Cultivars with both race-specific and race-nonspecific (adult plant) resistance are the most effective control method (10,36), despite the fact that the pathogen can overcome race-specific resistance in a few years. Therefore, it is important to identify new resistance sources in the seedling stage for specific resistance to reduce the amount of damage caused by the rust during the seedling stages.

The objective of this study was to identify *D. villosum* accessions resistant to the eyespot and stripe rust pathogens for potential use as sources of genes for resistance to these important diseases of wheat.

MATERIALS AND METHODS

Genetic stocks. A total of 219 accessions of *D. villosum* were obtained from the Western Regional Plant Introduction Station, United States Department of Agriculture-Agriculture Research Station (USDA-ARS), Washington State University, Pullman, and C. O. Qualset, University of California, Davis. A set of disomic addition lines of *D. villosum* (1V, 2V, 4V, 5V, 6V, and 7V) in a cv. Chinese Spring background (19,40) was obtained from the Wheat Genetics Resource Center, Kansas State University, Manhattan. A chromosome 4V substitution with chromosome 4D of cv. Yangmai-5 and a translocation line

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of 6VS/6AL of the same cultivar were provided by P. D. Chen, Cytogenetics Institute, Nanjing Agricultural University, Nanjing, China. The lines VPM-1 (resistant) and Chinese Spring (susceptible) were used as controls for eyespot reaction.

Eyespot reaction. A genetically modified strain of *Pseudocercosporella herpotrichoides,* expressing β-glucuronidase (GUS), was used as inoculum (11). A total of 219 accessions from four countries (Table 1) were screened for reaction to *P. herpotrichoides*. Two seedlings in each of three replicates were inoculated at the two-leaf stage with a suspension of 1×10^5 conidia/ml. Following incubation for 6 weeks in a growth chamber at 13 to 15°C and 98 to 100% relative humidity, the diseased portions of stems were harvested, visually evaluated for disease severity (45), and frozen at -20°C. Plants were rated visually for disease severity using a scale of 0 to 4, where 0 = no symptoms, 1 = a lesion only on the first leaf sheath, 2 = a lesion on the first leaf sheath and a small lesion on the second leaf sheath, 3 = a lesion on the first sheath and up to half of the second sheath, and 4 = a lesion covering the first and second sheaths (46). Sap was extracted from infected stem pieces with a Maxi-torq sap extractor (model 4Z522, Ravanel Specialities Company, Seneca, SC) with 2.5 ml of GUS extraction buffer per stem (22). GUS activity was measured fluorometrically following addition of 4-methylumbelliferyl β-D-glucoside (22). GUS scores were used to reflect the extent of colonization. Therefore, resistant lines have lower GUS scores than susceptible lines (11). Relative resistance was determined by comparing the disease reactions of individual lines with known resistant genotypes. All accessions were screened in a second experiment using the same method and design.

Stripe rust reaction. A total of 115 accessions were chosen randomly for evaluation of resistance to *Puccinia striiformis* (Table 1). Three races of *P. striiformis* representing most of the virulence genes present in the Pacific Northwest were used for inoculation (46). Two seedlings per accession in each of three replicates were uniformly inoculated with single-race cultures of urediniospores at the two-leaf stage and kept in environmental conditions optimum for disease development (46). Stripe rust infection type was recorded 15 to 18 days and 21 to 23 days after inoculation (46). Infection types were recorded as 2, 5, or 8, representing resistant, intermediate, and susceptible types, respectively (27). Resistant accessions were retested to confirm their resistance.

Statistical analysis. Homogeneity of error variance was confirmed and joint analysis of variance for the two experiments was carried out using a factorial arrangement of treatments (33) where "experiment" was considered as a random factor, "origin" as a fixed factor randomized within the experiment, and "accession" was nested and randomized within the origin with six total observations (replications) per accession. GUS data were transformed with a $\log_{10}$ transformation before analysis to remove a correlation between mean and variance. SAS statistical analysis software (SAS Institute, Cary, NC) was used for analysis of variance. Means were differentiated by Fisher's least significant difference (P = 0.05).

Table 1. Number of accessions of *Dasypyrum villosum* from each country of origin evaluated for resistance to *Pseudocercosporella herpotrichoides*, cause of eyespot, and three races of *Puccinia striiformis*, cause of stripe rust

	Stripe rust		Eyespot	
Country	**No. tested**	**Resistant**	**No. tested**	**Resistant**
Greece	32	27	51	51
Italy	69	5	131	131
Turkey	3	1	3	3
Yugoslavia	11	0	34	34
Total	115	33	219	219

Table 2. Combined analysis of variance of $\log_{10}$-transformed β-glucuronidase (GUS) activity of two experiments and origins of accessions

Origin	Source[a]	DF	Mean square	*F* value[b]	*Pr* > *F*
Combined	Experiment	1	1.67	11.6*	0.0115
	Ori	3	1.20	4.8	0.1147
	Acc (Ori)	212	0.11	1.4**	0.0055
	Exp × Ori	3	0.25	3.2*	0.0234
	Exp × Acc (Ori)	208	0.07	1.3*	0.0124
	Error	856	0.06		
	Total	1283			
Greece	Experiment	1	0.21	47.9**	0.0001
	Acc	50	0.03	1.9**	0.0010
	Exp × Acc (Ori)	50	0.06	1.3	0.1460
	Error	200	0.05		
	Total	301			
Italy	Experiment	1	0.86	13.5**	0.0003
	Acc	128	0.11	1.7**	0.0001
	Exp × Acc (Ori)	126	0.08	1.3*	0.0296
	Error	514	0.06		
	Total	769			
Turkey	Experiment	1	0.22	4.9*	0.0448
	Acc	1	0.02	0.04	0.8384
	Exp × Acc (Ori)	1	0.06	1.3	0.2696
	Error	14			
	Total	17			
Yugoslavia	Experiment	1	0.48	8.9**	0.0036
	Acc	33	0.12	2.1**	0.0014
	Exp × Acc (Ori)	31	0.06	1.2	0.2675
	Error	128	0.05		
	Total	193			

[a] Ori = origin, Acc = accessions, and Exp = experiment.
[b] Values followed by * or ** are statistically significant at $P < 0.05$ and $P < 0.01$, respectively.

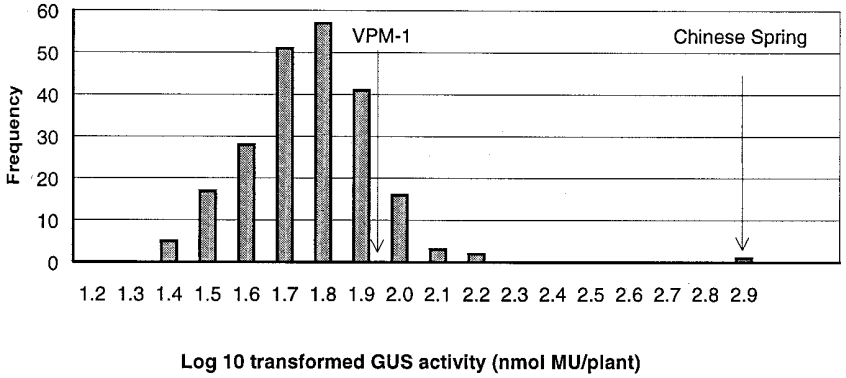

Fig. 1. Frequency distribution of $\log_{10}$-transformed β-glucuronidase (GUS) activity of 219 accessions of *Dasypyrum villosum* inoculated with a genetically modified strain of *Pseudocercosporella herpotrichoides* expressing GUS.

RESULTS

Differences in reaction to *Pseudocercosporella herpotrichoides* among accessions from different origins were not significant (Table 2); however, highly significant differences were observed among accessions within origins. The GUS scores for the population followed a normal distribution (Fig. 1) with a mean $\log_{10}$ GUS value of 1.71 and a standard deviation of $s = 0.27$ (Table 3). Of the accessions studied, 158 (72%) had statistically lower ($P = 0.05$) GUS scores than VPM-1 (Figure 1). In fact, six of the accessions from Greece (W6 7264, W6 7266, W6 7269, W6 7279, W6 7315, and W6 7316) and one accession from Italy (38-5) had about 25% lower GUS scores than VPM-1 ($P = 0.05$; Table 4). These accessions offer a potentially higher degree of resistance than *Pch*1. Only 21 accessions (9.5%) had GUS scores higher than VPM-1, and 40 accessions (18%) fell into the same class with VPM-1. Among four different geographical areas, accessions collected from the former Yugoslavia had the highest mean GUS scores (less resistant), and Greece had the most resistant accessions (Tables 3 and 4). As reported earlier (35), the CS+4V addition line was resistant to eyespot and all the other disomic addition lines, and the 4V substitution line were susceptible (Table 4).

Accessions of *D. villosum* showed a diverse reaction to *Puccinia striiformis*

Table 3. Means and standard deviations (SD) for $\log_{10}$-transformed β-glucuronidase reporter gene activity for experiments and origins with relative number of observations.

Origin	No. of observations	Mean	SD
Greece	302	1.64	0.26
Italy	770	1.71	0.27
Turkey	18	1.68	0.25
Yugoslavia	194	1.81	0.26
Total experiments	642	1.71	0.27
Control cultivars			
Chinese Spring	6	2.92	0.31
VPM-1	6	1.98	0.15

Table 4. *Dasypyrum villosum* accessions and wheat genotypes resistant to one or more North American races of *Puccinia striiformis* and *Pseudocercosporella herpotrichoides*

							GUS activity (nmol MU/plant)[a]	
Accession or genotype	Country of origin	CDL 17[b]	CDL 43	CDL 45	Eyespot	Visual rating	First	Second
W6 7264	Greece	R	R	S	R	1.0	1.47	1.55
W6 7266	Greece	R	I[3]	R	R	1.0	1.37	1.55
W6 7267	Greece	R	R	I	R	1.0	1.60	1.85
W6 7268	Greece	R	R	R	R	1.0	1.60	1.59
W6 7269	Greece	R	R	S	R	1.0	1.28	1.57
W6 7274	Greece	R	R	R	R	1.0	1.47	1.66
W6 7278	Greece	I	R	R	R	1.0	1.30	1.42
W6 7279	Greece	S	I	R	R	1.0	1.45	1.49
W6 7280	Greece	S	R	I	R	1.0	1.60	1.88
W6 7281	Greece	R	ND	R	R	1.0	1.46	1.82
W6 7282	Greece	S	R	S	R	1.0	1.42	1.80
W6 7285	Greece	S	R	S	R	1.0	1.51	1.64
W6 7288	Greece	R	R	S	R	1.0	1.64	1.53
W6 7292	Greece	I	R	S	R	1.0	1.74	1.60
W6 7293	Greece	R	R	R	R	1.0	1.51	1.71
W6 7296	Greece	R	R	S	R	1.0	1.76	1.70
W6 7297	Greece	R	I	R	R	1.0	1.67	1.72
W6 7300	Greece	S	R	S	R	1.0	1.81	2.08
W6 7301	Greece	R	R	R	R	1.0	1.28	2.05
W6 7302	Greece	ND	R	I	R	1.0	1.24	1.77
W6 7304	Greece	R	R	R	R	1.0	1.66	1.56
W6 7307	Greece	S	R	S	R	1.0	1.62	1.86
W6 7310	Greece	R	I	R	R	1.0	1.44	1.78
W6 7312	Greece	I	R	R	R	1.0	1.92	1.97
W6 7314	Greece	S	R	S	R	1.0	1.61	1.56
W6 7315	Greece	R	R	R	R	1.0	1.50	1.44
W6 7316	Greece	R	R	R	R	1.0	1.26	1.50
17-9C	Italy	R	R	R	R	1.0	1.86	2.01
38-5	Italy	R	I	I	R	1.0	1.28	1.41
70-1	Italy	R	I	S	R	1.0	1.67	1.87
137-6	Italy	R	I	S	R	1.0	1.69	1.67
138-B	Italy	R	I	I	R	1.0	ND	ND
PI 470279	Turkey	S	R	S	R	1.0	1.61	1.72
Chinese Spring		S	R	R	S	3.3	3.19	2.66
4V(4D) Sub.[c]		S	S	S	S	4.0	3.33	2.56
VPM-1		ND	ND	ND	R	1.0	1.93	2.02
CS+1V		S	S	S	S	4.0	3.66	2.88
CS+2V		S	S	S	S	3.7	2.82	2.54
CS+4V		S	S	S	R	2.0	1.81	1.95
CS+5V		S	S	S	S	3.8	3.11	2.72
CS+6V		S	S	S	S	4.0	3.10	2.75
CS+7V		S	S	S	S	4.0	3.38	2.56
Tran. (6VS/6AL)[d]		R	R	R	ND	ND	ND	ND
Yangmai-5		S	S	S	ND	ND	ND	ND

[a] GUS = β-glucuronidase reporter gene; activity for first and second experiment.
[b] CDL = Cereal Rust Laboratory designations. R = resistant, S = susceptible, I = intermediate, and ND = undetermined.
[c] *Triticum aestivum* cv. Yangmai-5–*D. villosum* chromosome 4V(4D) substitution line.
[d] *T. aestivum* cv. Yangmai-5–*D. villosum* chromosome 6VS/6AL translocation line.

(Tables 1 and 4). A total of 33 accessions (28.6%) had a race-specific reaction to one or more CDL races, 13 (11%) were intermediate, and 8 (7%) were resistant to all three races. Most of the resistant accessions originated from Greece. Accessions from the former Yugoslavia were susceptible to all races. Only five accessions (7%) from Italy had resistance to one or more stripe rust races. The 6VS/6AL-translocation line was resistant to all races, whereas the other disomic addition lines of *D. villosum*, the substitution line, and the wheat parent Yangmai-5 were susceptible (Table 4).

DISCUSSION

We have identified at least one new resistance gene for *P. striiformis*, designated *Yr*26 (30), which is located on the short arm of chromosome 6V of *D. villosum*. The fact that none of the previously named stripe rust resistance genes (*Yr*1 to *Yr*18) were located on group 6 chromosomes of wheat (31) and the homology of *D. villosum* chromosomes with wheat chromosomes (13,34,47) indicates that this is a new gene. Recently, seven stripe rust resistance genes were located on group 6 chromosomes of wheat through monosomic analysis (7–9). However, confirmation of the location and uniqueness of these seven genes requires further analysis. The 6VS/6AL-translocation line also expresses powdery mildew resistance (6). The presence of two important disease-resistance genes, the lack of reported negative-linkage drags, and the stable transmission of the translocated arm of chromosome 6V in wheat may allow for successful utilization of this germ plasm in breeding programs.

There was no difference in reaction to *Pseudocercosporella herpotrichoides* between the two experiments in terms of visual scores; however, the difference in GUS scores was significant. This may be due to the quantitative nature of the disease screening method, micro- and macro-environmental effects, or differences in genetic constitutions of each accession. *D. villosum* is an outcrossing species; therefore, high genetic variation is expected. Based on our results, genetic diversity within population (origins) was larger than among populations. Greater genetic variation within populations than among populations in natural populations of *D. villosum* has also been reported for high-molecular-weight glutenin protein subunits (47), seven quantitatively controlled morphological traits (48), and isozymes and ribosomal RNA loci (14).

The fact that all of the accessions were resistant to *P. herpotrichoides* may be a disadvantage for genetic studies of the eyespot resistance gene or genes in *D. villosum*. The lack of susceptibility among the accessions, for example, could make mapping and tagging of this gene difficult. However, the susceptible 4V substitution line, identified here, was crossed with the resistant CS+4V addition line and the resistance gene was mapped in a wheat background after confirming that the substituted chromosome was, in fact, 4V (45).

Pch1, transferred from *A. ventricosa* through the genotype VPM-1, has been the most utilized resistance gene for eyespot in breeding programs worldwide (1,2,16,25, 29,32,42). The first eyespot-resistant cultivars in the United States, Madsen and Hyak, were produced using VPM-1 (1,2). These cultivars have had a great impact on Pacific Northwest wheat production. During the 1992 to 1993 growing season, cv. Madsen was planted on about 400,000 ha in Washington State and savings to the wheat industry were estimated at $6,000,000 (T. D. Murray, *unpublished*) as a result of reduced fungicide application. Despite the success of cvs. Madsen and Hyak, the *Pch1* gene does not provide complete resistance to *P. herpotrichoides*. Based on a 12-year eyespot field-evaluation test, cv. Madsen still sustained significant yield loss (24). Producing a winter wheat cultivar with a greater level of resistance to eyespot than cvs. Madsen and Hyak may require pyramiding of eyespot resistance genes. Availability of the *D. villosum* resistance gene, along with other recently identified genes in *T. tauschii* (46) and in *T. monococcum* (5), may enable breeders to pyramid multiple genes for resistance to eyespot in a single cultivar. Incorporating multiple resistance genes into a single genotype may be accomplished through the use of marker-based selection for each gene, such as the endopeptidase isozyme band that was used in the production of cvs. Madsen and Hyak.

To continue advances in wheat production while reducing the need for chemical control, new sources of disease resistance genes must be exploited. The identification of new genes for resistance reported here should be of benefit to breeding programs working with these two diseases.

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